World Journal of Biology Pharmacy and Health Sciences

eISSN: 2582-5542

Cross Ref DOI: 10.30574/wjbphs

Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Physical quality, total lactic acid bacteria and *Escherichia coli* in pork fermented with roasted black glutinous rice (*Oryza sativa* var. glutinosa)

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World Journal of Biology Pharmacy and Health Sciences, 2026, 27(01), 172–178

Publication history: Received on 11 June 2026; revised on 25 July 2026; accepted on 28 July 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.27.1.0396

Abstract

Wadi is a traditional Indonesian fermented product generally made using rice as a carbohydrate source. The use of roasted black glutinous rice has the potential to improve fermentation quality because it contains starch, anthocyanins, and phenolic compounds that can support the activity of fermentative microorganisms. This study aims to analyze the effect of adding roasted black glutinous rice on the physical characteristics and microbiological safety of pig wadi fermented in bamboo. The study used a Completely Randomized Design with four treatments, namely P0 (white rice), P1 (10% roasted black glutinous rice), P2 (20% roasted black glutinous rice), and P3 (30% roasted black glutinous rice), each repeated four times. Observed variables included pH, water holding capacity, raw loss, cooking loss, total lactic acid bacteria, and the presence of *Escherichia coli*. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's test at a significance level of 5%. The results showed that the addition of roasted black glutinous rice had a significant effect ($P<0.05$) on the pH value, but did not significantly affect ($P>0.05$) on the water holding capacity, raw loss, and cooking loss. The pH value ranged from 5.77–6.35, water holding capacity 66.52–70.11%, raw loss 3.62–6.93%, and cooking loss 25.26–29.79%. Total lactic acid bacteria increased with increasing concentration of roasted black glutinous rice with the highest number in treatment P3 of 2.02×10^8 CFU/g, while *E. coli* was not detected in all treatments. Increasing the concentration of roasted black glutinous rice was able to support the growth of lactic acid bacteria without reducing the physical quality of the product, thus producing pig wadi with good microbiological safety.

Keywords: Lactic acid bacteria; Roasted black glutinous rice; Fermentation; Physical quality; Pig wadi

1. Introduction

Meat is an animal product that is susceptible to spoilage because it serves as a favorable medium for microbial growth. This is addressed through product diversification. One example is a fermented product called wadi. Wadi is a traditional product popular among the Dayak people of Kalimantan. It is generally made from fish, salted, and samu (ground rice or glutinous rice), which is roasted and then stored in bamboo [3].

Samu is typically made from rice, but in this study, black glutinous rice was made. Black glutinous rice (*Oryza sativa* var. glutinosa) contains bioactive compounds, including antioxidants that can function as natural preservatives, such as anthocyanins and flavonoids. Anthocyanins and flavonoids are antioxidants, and black glutinous rice is found in higher concentrations than white rice.

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[4] reported that the darker the rice, the higher its antioxidant pigment content, which is a natural pigment, such as anthocyanins, which are part of the flavonoid group. Anthocyanins and flavonoids play a role in protecting the body from premature aging, contributing significantly to preventing blood vessel blockages and maintaining heart health, making them a functional food. [22] reported that adding antioxidants to meat serves to prevent lipid oxidation, inhibit the development of unpleasant odors, and improve color stability. According to [21], using 20% samu and 10% salt for a 7-day fermentation period produced the best-quality fermented meat, with a pH of 5.39, cooking loss of 36.33%, and a total Lactic Acid Bacteria (LAB) count of 4.19×10^6 CFU/g.

While the meat is in the bamboo, biodegradation occurs. This is due to the microbes present in the bamboo used to make wadi. [24] reported that bamboo contains lactic acid bacteria (Lactobacillus species *Lactobacillus plantarum*) which play a role in the fermentation process by converting carbohydrates into lactic acid. [14] reported that lactic acid contributes to improving texture and flavor, and is also effective in inhibiting the growth of pathogenic bacteria such as *E. coli* and S. aureus.

[23] stated that Lactic Acid Bacteria (LAB) have the potential to affect the physical stability of meat by altering its water-holding capacity. Meat with a low pH tends to have a lower water-holding capacity, thus increasing fluid loss during storage (raw loss) and cooking (cooking loss). In addition to Lactic Acid Bacteria (LAB), there is also yeast that lives in bamboo segments [17], which functions to produce lipase enzymes that play a role in hydrolyzing fat so that it can be used as a source of carbon and energy in the form of fatty acids [29].

Based on the description, this study was conducted to evaluate the effect of adding roasted black glutinous rice (*Oryza sativa* var. glutinosa) to pork fermentation on physical quality including water holding capacity, pH, raw loss, and cooking loss, as well as on total lactic acid bacteria and *Escherichia coli*.

2. Materials and Methods

2.1. Material

The main ingredients used were Landrace pork loin, black glutinous rice (*Oryza sativa* var. glutinosa), and 10% salt by weight. Materials for physical quality analysis included pH 4 and pH 7 buffer solutions, distilled water, filter paper, and plastic bags. Microbiological analysis used de Man Rogosa Sharpe Agar (MRSA) for lactic acid bacteria (LAB) enumeration, Eosin Methylene Blue Agar (EMBA) for *Escherichia coli* detection, 0.85% physiological NaCl solution, 70% alcohol, and other supporting microbiological materials.

The main equipment used included an analytical balance, pH meter, centrifuge, water bath, autoclave, incubator, vortex mixer, sterile petri dishes, micropipettes, oven, graduated cylinders, Erlenmeyer flasks, test tubes, and other laboratory glassware.

2.2. Methods

This research was conducted over two months using a Completely Randomized Design (CRD) with four treatments and four replications, resulting in 16 experimental units. Each experimental unit used 250 grams of pork, 10% salt, and varying concentrations of roasted black glutinous rice.

- P0: 250 grams of landrace pork + 10% salt + 20% roasted white rice (control)
- P1: 250 grams of landrace pork + 10% salt + 10% roasted black glutinous rice
- P2: 250 grams of landrace pork + 10% salt + 20% roasted black glutinous rice
- P3: 250 grams of landrace pork + 10% salt + 30% roasted black glutinous rice

2.2.1. Making pork wadi

The pork loin is cut into 250g pieces, washed with clean water, and then drained. The meat is then coated with salt, equal to 10% of its weight, and left to marinate for 24 hours. Black glutinous rice is roasted at 70°C for 25 minutes until it turns blackish brown, then ground and sieved to obtain a fine flour. After the salting process is complete, the meat is mixed with white rice flour and roasted black glutinous rice according to the treatment (20%, 10%, 20%, and 30%) until homogeneous. The mixture is then placed in bamboo sections, covered with aluminum foil to create semi-anaerobic fermentation conditions, and fermented at room temperature for seven days.

2.2.2. Observed Variables

Observed variables included the physical and microbiological quality of the fermented product.

Water Holding Capacity

Water holding capacity (WHC) was analyzed using the centrifugation method according to [23]. A 2 g sample was centrifuged at 3,600 rpm for 60 minutes, then calculated based on the percentage of water retained by the meat tissue.

pH Value

The pH value was measured using a pH meter calibrated with pH 4 and pH 7 buffer solutions according to the [2] method. The electrode was dipped into the sample, and the pH value was read directly.

Raw loss

raw loss was analyzed according to the [23] method. Samples were weighed before storage and then hung at room temperature in sealed plastic bags. After the test period was completed, the samples were reweighed and the percentage of raw loss was calculated based on the difference in weight before and after treatment.

Cooking Loss

Cooking loss was analyzed using the [23] method. Samples were weighed, then placed in heat-resistant plastic bags and cooked in a water bath at 80 °C for 60 minutes. After cooling and drying, the samples were reweighed to calculate the cooking loss percentage.

Total Lactic Acid Bacteria

Total LAB were analyzed using the Total Plate Count (TPC) method on MRSA media according to [12]. Samples were serially diluted to 10^{-7}, then inoculated using the pour plate method and incubated at 37°C for 24 hours. Results were expressed as colony-forming units per gram (CFU/g).

Total *Escherichia coli*

Escherichia coli analysis was performed using EMBA selective media in accordance with the Indonesian National Standard [6]. Samples were serially diluted, then inoculated using the spread plate method and incubated at 37°C for 24 hours. Metallic green colonies were counted as *E. coli* colonies

3. Results and discussion

Table 1 Physical Quality, Total Lactic Acid Bacteria, and *E. coli* of Pig Wadi Fermented with Roasted Black Glutinous Rice

Variables	**Treatment 1)**				**SEM 2)**
	P_0	**P_1**	**P_2**	**P_3**	
Water Holding Capacity (%)	66.52 ^{a}	68.06 ^{a}	70.11 ^{a}	69.97 ^{a}	3.191
pH Value	5.80^{b}	6.35^{a}	5.96$^{ab\ 3)}$	5.77^{b}	0.150
Raw Loss (%)	6.93^{a}	6.25^{a}	3.72^{a}	3.62^{a}	1.396
Cooking Loss (%)	25.26 ^{a}	29.79 ^{a}	29.02 ^{a}	26.96 ^{a}	2.742
Total Lactic Acid Bacteria CFU/g	1.43×10^{8}	1.36×10^{8}	1.41×10^{8}	2.02×10^{8}	
Total *Escherichia coli* CFU/g	-	-	-	-	

Note: 1) P0: 250 grams of landrace pork + 10% salt + 20% roasted white rice (control); P1: 250 grams of landrace pork + 10% salt + 10% roasted black glutinous rice; P2: 250 grams of landrace pork + 10% salt + 20% roasted black glutinous rice; P3: 250 grams of landrace pork + 10% salt + 30% roasted black glutinous rice; 2)SEM: Standard Error of the Treatment Mean; 3) (a,b) : Different letters in the same row indicate significant differences (P<0.05)

The results of the physical quality analysis (pH, raw loss, cooking loss, water holding capacity) of pig wadi fermented with roasted black glutinous rice are shown in Table 1.

3.1. Water Holding Capacity

Water holding capacity (WHC) is the ability of meat to bind and retain water in its muscle tissue, both during storage and processing processes such as heating. The water holding capacity of fermented pork wadi with roasted black glutinous rice showed an increase but was not significantly different (P <0.05) in all treatments. This is because the higher the concentration of roasted black glutinous rice added, the pork wadi fermented in bamboo was not able to be biodegraded by LAB so it was not able to denature meat proteins.

Black glutinous rice has a high amylopectin content (98% of total starch), so LAB is less able to penetrate protein gel cells, which causes no change in the protein's ability to bind meat [31]. Water holding capacity is influenced by pH. In this study, the pH obtained was above the isoelectric point, [18], reported that the isoelectric point of meat proteins ranges from (5.0-5.4), so it is not optimal causing denaturation of myofibril proteins in meat which causes the binding ability of meat to be strong. This is in line with research by [13] who reported that the water-binding capacity of sui wu'u meat ranges from 20% to 28% with a pH value ranging from 4.0 to 1. This research result is also supported by [19] who reported that the relatively neutral pH of meat (5.8–6.0) allows myofibrillar proteins to maintain an optimal three-dimensional structure for water binding, thus stabilizing their water-binding capacity. Previous studies reported that, small changes in pH far from the protein's isoelectric point will not cause significant changes in water retention capacity [1].

3.2. pH Value

The results of the analysis of variance showed that the addition of roasted black glutinous rice had a significant effect (P<0.05) on the pH value of the pork wadi. The pH ranged from 5.77 to 6.35, with the highest value obtained in treatment P1 (10% roasted black glutinous rice), while the lowest value was obtained in treatment P3 (30% roasted black glutinous rice). Treatment P1 had a higher pH value than P0, while treatment P2 was not significantly different from either P0 or P3.

The high pH value of the pig's wadi in treatment P1 is thought to be due to the lower availability of carbohydrate substrate for LAB to utilize as an energy source compared to the other treatments. Furthermore, the roasted black glutinous rice used in this fermentation has a more complex starch structure compared to P0, which uses white rice with a simpler starch structure. The starch in black glutinous rice is dominated by amylopectin with a complex branched structure, making it more difficult for fermentation microorganisms to degrade rapidly [27] This is supported by the lower total LAB in P1 (1.36×108) and P2 (1.41×108) compared to P0 (1.43×108).

Although black glutinous rice starch has a complex structure that is difficult to degrade, at higher concentrations (P3), the amount of available substrate remains greater. Consequently, some of the hydrolyzed starch can become an energy source for LAB in greater quantities, thereby increasing lactic acid production and lowering pH. This is supported by research by [7], which states that complex starch in 29 cereals undergoes gradual degradation into simple sugars during fermentation, which are then utilized by LAB.

3.3. Raw Loss

Raw loss in fermented meat (often referred to as "raw loss" or "part of maturation loss") is the weight loss of meat products that occurs during the fermentation and cooking (ripening/drying) processes in the raw state, primarily due to the reduction in air content (evaporation) and the decomposition of meat components. Raw loss values in this study ranged from 3.62% to 6.94% and showed no significant differences (P>0.05) across all treatments. This is likely because the pH values between treatments remained relatively close (5.78–6.35), thus not causing changes in the charge and structure of myofibrillar proteins.

Raw loss is closely related to the ability of muscle tissue to retain water during storage. [23] stated that fluid loss in raw meat is significantly influenced by pH and protein integrity. If the pH change does not approach the protein isoelectric point, drip loss will not significantly increase.

Research by [28] on fish wadi reported that varying the concentration of salt did not significantly affect air content and weight loss during the early stages of fermentation. This is also supported by [8]. who stated that fermentation with lactic acid bacteria does not always significantly affect drip loss if the final product pH remains within a stable range.

3.4. Cooking Loss

Cooking loss for fermented meat is the percentage of weight loss caused by the release of liquid (meat juice) during the cooking process (boiling/frying). This loss is influenced by changes in protein structure and a decrease in pH during fermentation.

Cooking loss values in this study ranged from 25.26% to 29.80% (Table 1). Statistically, cooking loss for pork wadi was not significantly different ($P>0.05$) across all treatments. This is because cooking loss is closely related to water holding capacity (WHC), where the higher the meat's water-holding capacity, the less water loss during the cooking process. In this study, the water holding capacity values did not show significant differences between the treatments, thus directly influencing the cooking loss results, which were also not significantly different. This indicates that the protein structure of the meat in each treatment was relatively stable and had not undergone significant changes [23] stated that cooking loss is influenced by the protein's ability to retain water. This is thought to be influenced by the role of roasted black glutinous rice, which contains high levels of amylopectin. Amylopectin can absorb and retain water and function as a filler in the meat protein matrix, thereby helping maintain the stability of muscle tissue structure. [31] stated that starch with a high amylopectin content has the ability to form stable gels and increase water retention in food systems.

Furthermore, the pH value in this study was in the range of 5.77–6.35, which is still above the protein's isoelectric point. This condition allows the protein to retain its water-binding capacity well, so that fluid loss during cooking does not increase significantly. According to [26], changes in cooking loss in fermented products will not be significant if the pH and protein structure are relatively stable. These results also align with [8], who stated that fermentation by lactic acid bacteria does not always have a significant effect on cooking loss if there are no significant changes in the water-binding capacity and protein structure of the meat. Based on meat quality standards, a good cooking loss value is in the range of 15–40% [23]. Thus, the cooking loss value in this study was still within the normal range, which indicates that the fermented pork wadi product has good physical quality and is able to maintain water content during cooking.

3.5. Total Lactic Acid Bacteria (LAB)

The total LAB in pig wadi in this study was obtained at 1.36×10^8 CFU/g - 2.02×10^8 CFU/g (Table 1), in the P0 – P1 treatment there was a decrease (1.36×10^8 CFU/g-1.43×10^8 CFU/g), this decrease is thought to be because in P1 with the provision of 10% black glutinous rice causing less available substrate for LAB compared to P0 with the provision of 20% white rice, while in P2 and P3 there was an increase to (1.41×10^8 CFU/g - 2.02×10^8 CFU/g). This is because the provision of 20% and 30% black glutinous rice causes more available substrate for LAB. The increase in the number of LAB indicates that although black glutinous rice starch is dominated by amylopectin which has a complex branched structure and is relatively difficult to degrade directly, causing LAB growth to continue. This is thought to be due to the presence of other microorganisms in the fermentation system that have amylolytic activity, so they are able to hydrolyze some of the starch into simple sugars which are then utilized by LAB as an energy source, this is supported by research by [17] who reported that there is yeast in bamboo segments.

The fermentation process carried out in bamboo containers also plays an important role in supporting the growth of LAB. Bamboo not only functions as a container, but also as a source of natural microorganisms. This is in line with research by Sugitha and [24] who reported that bamboo contains lactic acid bacteria, so the possibility of no decrease in pH is also caused by proteolytic bacteria found in bamboo and dominating in the initial phase of fermentation, proteolytic bacteria produce protease enzymes that break down meat proteins into amino acids, then the amino acids undergo deamination and produce ammonia (NH_3) which is basic, so it is able to neutralize some of the organic acids from fermentation and cause the pH to increase or decrease slowly [25]. After the LAB population develops more dominantly, then lactic acid is produced more so that the pH decreases gradually.

3.6. *Escherichia coli*

Analysis showed that there was no growth of *E. coli* in pig wadi fermented with roasted black glutinous rice. This is because during fermentation in bamboo, LAB biodegrades carbohydrates, creating acidic conditions. This acidic condition inhibits the growth of *E. coli* bacteria. [11] reported that *E. coli* bacteria are acid-resistant bacteria. In addition, black glutinous rice contains several bioactive components that function as antimicrobials such as anthocyanins, flavonoids, phenolic compounds, and amylofectin. [15] reported that *E. coli* is a sanitation indicator bacteria. Sanitation is an effort to prevent microbial contamination such as *E. coli* and Salmonella, through the application of hygiene [30], so this study has implemented a sanitation system. The same thing was also reported by [20] found that *E. coli* is a gram-negative bacterium that is sensitive to acidic environmental conditions. This is evident from the pH values obtained in this study (5.77-6.35) (Table 1).

In this study, wadi production followed Indonesian National Standards (SNI) standards. SNI (2000) [5] states that the standard for *E. coli* in food is around 10^1. Furthermore, the presence of bioactive compounds such as anthocyanins and polyphenols in black glutinous rice also plays a role in creating a selective environment that can inhibit the growth of competing pathogenic microorganisms, allowing LAB to grow more dominantly. This is supported by [9], who stated that the combination of LAB activity and bioactive compounds can increase microbiological stability through the production of organic acids and bacteriocins. Research by [16] also reported that LAB, such as *Lactococcus lactis*, has antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, thus the presence of high levels of LAB contributes to increased product microbiological safety. According to [10], during fermentation, LAB produces organic acids which lower the environmental pH, thereby creating conditions that are unfavorable for the growth of putrefactive microorganisms and pathogenic bacteria.

4. Conclusion

Wadi babi fermented with roasted black glutinous rice affected the physical quality (pH) but not the raw loss, cooked loss, or water holding capacity. It increased the total Lactic Acid Bacteria (LAB) (1.36×108-2.02×108) and did not show any *E. coli* growth. The 30% roasted black glutinous rice treatment with wadi babi was the best treatment, with a physical quality of pH (5.77), raw loss (3.62), total LAB (2.02×108), and no *E. coli* growth.

Compliance with ethical standards

Disclosure of conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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